COLLECTION, PRESERVATION AND DESPATCH OF APPROPRIATE DIAGNOSTIC SAMPLE FOR FIELD VETERINARIANS

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The starting point for the laboratory investigation of an animal disease is the taking of samples. Samples may be taken from animals or the environment for a variety of purposes, such as disease diagnosis, disease surveillance, health certification or monitoring the response to treatment or vaccination. A reliable laboratory report as an aid in the diagnosis of disease depends upon the care and thought used in the collection, handling, and transport of specimens.

1. COLLECTION OF SAMPLES

Before taking samples, careful consideration should be given to the purpose for which they are required. This will determine the type and number of samples needed to provide valid results. When samples are taken from live animals, care should be taken to avoid injury or distress to the animal or danger to the operator and attendants. It may be necessary to use mechanical restraint, tranquillisation or anaesthesia. Whenever handling biological material, from either live or dead animals, the risk of zoonotic disease should be kept in mind and precautions taken to avoid human infection (OIE Terrestrial Manual, 2008). Post-mortem examinations should be carried out under as aseptic conditions as is practicable. Care should be taken to avoid environmental contamination, or risk of spread of disease through insects or fomites. Arrangements should be made for appropriate safe disposal of animals and tissues. Considerable skill and care are required to decide on the correct samples to be sent to the laboratory. The samples collected should be representative of the condition being investigated and the lesions observed. Also the stage of the disease and lesion development should be considered, as well as the type of test(s) that will be performed. Frequently, a combination of blood samples for serology and tissues from dead or culled animals for microbiological culture and pathological examination will be required.

1. Sample collection from live animals

a) Blood- Blood samples may be taken for haematology or for culture and/or direct examination for bacteria, viruses, or protozoa, in which case it is usual to use anticoagulants, such as ethylene diamine tetra-acetic acid (EDTA) or heparin. They may also be taken for serology, which requires a clotted sample. Blood plasma is also used for some procedures. A blood sample is taken, as cleanly as possible, by venipuncture. In most large mammals, the jugular vein or a caudal vein is selected, but brachial veins and mammary veins are also used. Vena cava veins are also used in pigs. In birds, a wing vein (brachial vein) is usually selected. Blood may be taken by syringe and needle or by needle and vacuum tube (not easy in delicate veins but convenient in strong veins). Small quantities of blood are conveniently obtained by pricking with a triangular, solid-pointed needle. Ideally the skin at the site of venipuncture should first be shaved (plucked) and swabbed with 70% alcohol and allowed to dry. For samples that are collected with

anticoagulant, thorough mixing, using gentle agitation only, is necessary as soon as the sample has been taken. It may also be necessary to make a smear of fresh blood on a microscope slide; both thick and thin smears may be prepared. For polymerase chain reactions, EDTA is the preferred anticoagulant. For serum samples, the blood should be left to stand at ambient temperature (but protected from excessive heat or cold) for 1-2 hours until the clot begins to contract. The clot can then be ringed round with a sterile rod and the bottles placed in a refrigerator at 4°C. After several hours, or overnight, the sample can be centrifuged at about 1000 g for 10-15 minutes and the serum can be decanted or removed with a pipette. In order to establish the significance of antibody titres, paired serum samples will often need to be collected 7-14 days apart. An alternative method for collecting and transporting blood that is to be used for serology is to place a drop of blood on to filter paper, the blood is dried at room temperature and the sample can then be shipped unrefrigerated. Contact the laboratory to enquire if this method of collection is validated for the required tests.

b) Faeces- At least 10 g of freshly voided faeces should be selected. Faeces for parasitology should fill the container and be sent to arrive at the laboratory within 24 hours. If transport times are likely to be longer than 24 hours, the sample should be sent on ice or refrigerated to prevent the hatching of parasite eggs. Screw top containers or sterile plastic bags should be used for shipment; avoid tubes with rubber stoppers as gas generated can result in blowing the stopper off the tube, ruining the integrity of the sample and contaminating other samples in the package. An alternative and sometimes preferable method is to take swabs from the rectum (or cloaca), taking care to swab the mucosal surface. The swabs should be visibly coated with faecal material; however, samples collected with a swab are inadequate for parasitology. Care should be taken when collecting swabs from small, delicate animals or birds to avoid injury to the animal; small swabs are commercially available that should be used. Swabs should be transported in appropriate transport medium. Faeces are best stored and transported at 4°C.

c) Skin- In diseases producing vesicular lesions, collect, if possible, 2 g of affected epithelial tissue as aseptically as possible and place it in 5 ml phosphate buffered glycerine or Tris-buffered tryptose broth virus transport medium at pH 7.6. Additionally, the vesicular fluid should be sampled where unruptured vesicles are present; if possible, vesicular fluid should be aspirated with a syringe and placed in a separate sterile tube. Plucked hair or wool samples are useful for surface-feeding mites, lice and fungal infections. Deep skin scrapings, using the edge of a scalpel blade, are useful for burrowing mites and, in birds, feather tips can be taken for detection of viral antigen where Marek's disease is suspected.

d) Genital tract and semen- Samples may be taken by vaginal or preputial washing, or by the use of suitable swabs. The cervix or urethra may be sampled by swabbing. Samples of semen are best obtained using an artificial

vagina or by extrusion of the penis and artificial stimulation. The sperm-rich fraction should be present in the sample and contamination by antiseptic washing solutions should be avoided. Specific transport media and conditions are often required.

e) Eye- A sample from the conjunctiva can be taken by holding the palpebra apart and gently swabbing the surface. The swab is then put into transport medium. Scrapings may also be taken on to a microscope slide. The handles of metal-handled swabs are useful for this, to ensure that sufficient cells are removed for microscopic examination. Mucopurulent nasal and lacrimal discharges are rarely useful.

f) Nasal discharge (saliva, tears)- Samples may be taken with dacron, cotton or gauze swabs, preferably on wire handles as wood is inflexible and may snap. It may be helpful if the swab is first moistened with transport medium. The swab should be allowed to remain in contact with the secretions for up to 1 minute, then placed in transport medium and sent to the laboratory without delay at 4°C. Long protected nasopharyngeal swabs should be used to collect samples for some suspected viral infections.

g) Milk- Milk samples should be taken after cleansing and drying the tip of the teat, the use of antiseptics should be avoided. The initial stream of milk should be discarded and a tube filled with the next stream(s), a sample of bulk tank milk can be used for some tests. Milk for serological tests should not have been frozen, heated or subjected to violent shaking. If there is going to be a delay in submitting them to the laboratory, preservatives can be added to milk samples that are being collected for serological testing. If necessary, milk for bacterial examination can be frozen.

2. Sample collection at post-mortem

Samples of tissue from a variety of organs can be taken at post-mortem. Detailed procedures for conducting a post-mortem examination and collecting samples are described in most pathology text books. Animal health personnel should be trained in the correct procedures for post-mortem examination of the species of animals with which they work. The equipment required will depend on the size and species of animal, but a knife, saw and cleaver will be required, and also scalpel, forceps and scissors, including scissors with a rounded tip on one blade, for opening intestines. A plentiful supply of containers and tubes of transport media appropriate to the nature of the sample required should be available, along with labels and report forms. Containers should be fully labelled with the date, tissue and animal identification. Special media may be required for transport of samples from the field. The operator should wear protective clothing: overalls, washable apron, rubber gloves and rubber boots. Additionally, if potential zoonotic diseases are being investigated, the post-mortem examination should be conducted in a biological safety cabinet; if this is not possible, an efficient face mask and eye protection should be worn. If rabies or transmissible spongiform encephalopathies (TSEs) are suspected, it is usual to detach the animal's head.

Tissues may be collected for

microbiological culture, parasitology, biochemistry, histopathology and/or immunohistochemistry, and for detection of proteins or genome nucleic acids. In addition buccal, oropharyngeal or rectal (cloacal) swabs may be collected. The person conducting the post-mortem examination should have sufficient knowledge of anatomy and pathology to select the most promising organs and lesions for sampling. Each piece of tissue should be placed in a fully labelled separate plastic bag or sterile screw-capped jar. Swabs should always be submitted in appropriate transport media. Sterile instruments should be used for collecting specimens for microbiological culture and care should be taken not to contaminate tissues with intestinal contents. Disinfectants should not be used on or near tissues to be sampled for bacterial culture or virus isolation. The tissues may be sent to the laboratory dry or in bacterial or virus transport medium, depending on the type of specimen and the examinations required; swabs should be sent in transport medium. After collection, the samples for microbiological examination should be refrigerated until shipped. If shipment cannot be made within 48 hours, the samples should be frozen; however, prolonged storage at -20°C may be detrimental to virus isolation. For histopathology, blocks of tissue not more than 0.5 cm thick and 1-2 cm long are cut and placed in neutral buffered 10% formalin, which should be at least ten times the volume of the tissue sample. For certain suspected diseases, larger portions of brain are required; the brain is sectioned using a sagittal cut, half is submitted fresh, on ice, and the other half is submitted in 10% buffered formalin. Store and pack formalin-fixed tissues separately from fresh tissues, blood and smears. Care should be taken to insure that formalin-fixed tissues are not frozen. Once fixed, tissues can be removed from formalin and, as long as they are kept moist and protected (e.g. by wrapping in formalin-soaked paper towels, then sealed in screw-capped jars), they can be forwarded to the laboratory without formalin.

Specimens Required For Laboratory Examinations

Infectious Diseases

A. Bacterial

1. Anthrax: In case of horse, pig, dog and cat, smears from sanguinous discharge/ excretion of natural orifices as well. Swab of blood (from the ear vein) or exudates. Pieces of skin or spleen for Ascoli's test (if putrefaction has set it).

2. Actinomycosis and Actinobacillosis: Pus smears from the deeper part of the lesions. Affected organs tissue both on ice and in 10% formal saline.

3. Bacillary haemoglobinurea: Portions of liver showing lesions on ice and in formal saline separately.

4. Black disease: Duplicate samples of necrotic portion of liver on ice and in formal saline (10%).

5. Black quarter and malignant oedema: Smears of haemorrhagic muscle exudates from a freshly dead animal. Portion of affected muscle, air dried (or on ice) and in formalin (10%) separately.

6. Botulism: Suspected food and intestinal contents on ice.

7. Brucellosis: Blood and serum samples. Quarter milk samples. In case of abortion, foetus, or foetal stomach contents and heart blood on ice.

8. Chronic Respiratory Disease (CRD): Affected live birds or dead birds packed on ice. Serum samples.

9. Enterotoxaemia: Smears from bowel mucous membrane and intestinal loop or samples of ingesta from duodenum, jejunum and ileum. Ingesta to be preserved with 3-4 drops of chloroform (formalin should not be used).

10. Fowl cholera: Affected birds (chronic cases). Heart blood, liver and spleen on, ice.
11. Fowl Spirochaetosis: Sick birds or blood smears from such birds.

12. Fowl typhoid: Heart blood, liver and spleen on ice or recently dead bird on ice.

13. Glanders: Swabs from nasal and skin lesions. Lungs and other organs showing lesions on ice and in formal saline (10%).

14. Johne's disease: Smears of rectal mucosa scrapings. From dead animals, portions of intestine showing lesions and associated lymph nodes on ice and in formal saline separately.

15. Leptospirosis: Portions of kidney and liver on ice and in formal saline (10%). Blood (collected during febrile stage of the disease) on ice. Freshly voided urine on ice. Serum samples.

16. Listeriosis: In the case of abortion, foetus, or foetal stomach and heart, on ice. In the cases of encephalitis (circling disease) or septicaemia, portions of brain, liver, spleen and kidney on ice and in formal saline separately.

17. Pasteurellosis, Haemorrhagic Septicaemia and Swine plague: Blood smears or exudates obtained with the help of sterile syringe and needle from oedematous swelling. Heart blood, portions of liver, spleen, kidney and lungs showing pneumonic lesions on ice. Long bones packed in charcoal. Pieces of lung and other affected organs in 10% formal saline.

18. Contagious Bovine or Contagious Caprine Pleuropneumonia (CBPP/CCPP): Blood, serum portions of affected lungs and associated lymph nodes on ice and also in formal saline (10%) pleural fluid.

19. Pneumonia due to other infectious agents: Portion of diseased lungs and associated lymph nodes on ice, portion of lung in glycerine saline and portion of diseased lung in formal 10% saline.

20. Pullorum disease: Blood sera from adult birds. Freshly dead chick or heart blood and liver, on ice. Pieces of affected organs in 10% formal saline.

21. Pyosepticaemia neonatorum (septicaemia in new born animals) and Paratyphoid in animals: Mesenteric lymph nodes and portions of kidney, spleen and liver on ice. Portion of bowel in formal saline (10%).

22. Swine erysipelas: In acute cases, heart blood, portions of liver, kidney and spleen on ice. In chronic cases, affected joints (without opening) on ice. Organs showing lesions in formal saline (10%) serum samples.

23. Tuberculosis: Portions of lesions unpreserved on ice or in 25% glycerine saline and in formal saline (10%).

24. Ulcerative lymphangitis and Caseous lymphadenitis: Swabs and smears from the lesions. In case of caseouslymphdenitis, pieces of affected organs and lymph nodes also on ice or 25% glycerine saline and in formal saline (10%).

B. Viral and Rickettsial

26. African horse sickness: Blood, spleen and lungs in 50% glycerine containing 0.5% sodium citrate and 0.5% carbolic acid. Blood in EDTA. Blood for serum samples.

27. Avian encephalomyelitis and Infectious porcine encephalomyelitis (Technen disease): Sick birds or animals. Brain and spinal cord in formal saline (10%) and buffered glycerine separately. Serum from convalescent cases.

28. Avian leucosis complex: Affected birds or portions of liver, spleen and nerve if involved and other organs showing lesions in formal saline.

29. Canine distemper: Pieces of liver and spleen on ice. Portions of urinary bladder, kidney, liver, lung and trachea in formal saline.

30. Encephalitides arthropod borne: St. Louis, Western equine, Eastern equine, Japanese-B, Venezulean, Russian spring summer and Louping ill: Serum from acute and convalescent cases. Defibrinated or oxalated blood, portions of brain including medulla and spinal cord from freshly dead carcasses in buffered glycerine as well as in formal saline.

31. Equine abortion: Portions of placenta and foetal organs (liver and spleen) in glycerine saline. 50% foetal stomach and heart blood on ice. Pieces of liver, spleen, lungs and trachea of foetus in formal saline.10% serum of the mare.

32. Foot and mouth disease, Vescicular stomatitis and Vesicular exanthema: Epithelium from the vesicles along with fluid in phosphate buffered glycerine and in saline tissue culture medium containing antibiotics. Paired sera samples at early and late stages. Pieces of oral tissue including tongue and meat in 10% formal saline.

33. Infectious bronchitis: Lungs including trachea, spleen and heart blood in buffered glycerine and on ice. Serum samples particularly from old cases. Lungs and trachea in formal saline (10%).

34. Infectious laryngotracheitis: Live birds in early stages of the disease or trachea including tracheal exudates (from early sacrificed case) in 50% glycerine saline. Portion of trachea in formal saline.

35. Pox in sheep, goats, pigs and cattle and Contagious pustular dermatitis (Contagious ecthyma) in sheep and goats: Scabs in 50% glycerine saline or unpreserved. Pieces of skin and other organs with lesion in 10% formal saline. Blood (at febrile stage).

36. Fowl pox: Scabs in 50% glycerine saline or unpreserved. Cutaneous lesion in 10% formal saline.

37. Pseudorabies: Brain and spinal cord in glycerine saline as well as in formal saline (10%).

38. Psittacosis and Ornithosis: Serum from acute and convalescent cases. Whole blood on ice. Dead or sacrificed bird wrapped with Lysol soaked cloth and packed over ice. Pieces of affected organs in 10% formal saline.

39. Rabies: Head packed over ice. Brain half of it, divided longitudinally in formal saline 10% and half in 50% glycerine saline.

40. Ranikhet disease (New Castle disease): Freshly dead carcase packed over ice, or brain, spleen and liver in glycerine saline.

41. Rinderpest and Mucosal disease complex: Defibrinated or citrated heparinised blood when the animal is running high temperature. Pieces of lymph nodes in mesenteric tonsils and spleen on ice or in buffered glycerine. Pieces of spleen lymph node, intestine, oral mucosa, liver, lungs and kidney in 15% buffered saline or Bouin's fluid.

42. Swine fever: Defibrinated blood from living animals. Heart, blood, liver, tonsils and pancreas and spleen on ice, Organs (including brain) showing lesions in formal saline. 10%

43. Swine influenza and Viral pneumonia of pigs: Portion of lung showing pneumonia patch on ice, in buffered glycerine (50%) and in formal saline (10%) separately. Material should be collected after destroying the animal in early stage of the disease.

44. Blue tongue: Blood in heparin or OCG, Heart blood, bone marrow, pieces of spleen, liver on ice. Serum sample paired skeletal muscles and heart muscle in 10% formalin.

45. Peste – des - petits ruminants (PPR): Eye, mouth and rectal swabs on ice, about 10 ml. or more blood at the height of body temperature in anti coagulant. Pieces of spleen (10- 30 gm.), small intestine, mesenteric lymph nodes and lung on ice or buffered glycerine.Pieces of affected lung, spleen, small intestine (Payer's patches), mesenteric lymph nodes in 10% formalin saline. Materials from 5 or 6 or more animals be collected and dispatched for better picture of diseases/ outbreaks.

C. Parasitic

46. Anaplasmosis: Thin blood films, citrated or oxalated blood from sick animals.

47. Coccidiosis: Faeces in formalin and for in pot. Dichromate solution. Portion of intestine showing lesions in formal saline.

48. Piroplasmosis: Thin blood smears, heart blood, spleen and kidney smears from dead animals. Citrated or oxalated blood.

49. Theileriosis: Thin blood films. Smears or biopsy material from prescapular lymph node. Portions of lymph node, liver and kidney on ice and in formal saline 10%.

50. Trichmoniasis: Uterine discharge (collected within 24 hours of abortion or during heat period) on ice.

51. Trypanosomiasis: Citrated blood. Blood films. In camel, cattle and buffaloes, serum as well.

52. Parasitic infections: Faecal samples in formalin. From dead animals, unpreserved stomach or abomasum and small and large intestine, if possible, otherwise their contents in formalin. In case of lambs, kids, piglets and fowls, whole carcass or sick animal itself.

53. Mange: Scabs and deep skin scrapings.

D. Fungal

54. Fungal diseases (in general): Portions of organs/tissues showing lesions on ice and in formal saline 10% separately.

55. Aspergillosis of fowls (Brooder's pneumonia): Affected birds. Lungs along with caseous masses from the air sacs over ice. Lungs in formal saline 10%.

56. Ring worm: Skin scrapings from the lesions, including some hair roots, unpreserved in a tightly stopper container.

2. PRESERVTION OF SAMPLE

Preservation of materials for specific examination

Specific examination	Preservatives used
Bacteriological/ Mycological	• Blood and exudates: are to be collected aseptically with sterile Pasteur pipettes/syringes, and then put in sterile tubes or vials without any preservative and to be transported over ice. Especially for Anthrax and HS blood should be collected in sterile container /Cryo vials with (500-1000µl EDTA is sufficient) and without EDTA. After collection cotton soaked with alcohol should be placed on the collection site and burned and transported strictly under cold chain. • Tissues: Pieces of affected organs with lesions should be collected in sterile condition and should be transported under cold chain.
Virological	• Blood and exudates: to be collected aseptically without any chemical preservative and to be stored and transported on ice. In some viral infections (e.g. Blue Tongue and Classical Swine Fever blood may be collected in vials/vacutainer containing EDTA). • Tissues: Pieces of affected organs are to be collected under aseptic condition and transported either on ice. For FMD 50% buffered glycerine saline is the preferred transport medium
Parasitological	For identification of parasite and helminth ova 10% formalin is the preferred transport medium to preserve the integrity of the ova.. For coccidial oocysts, 2.5% Potassium dichromate solution is the preferred transport medium.
Serological tests:	• After collection, serum samples have to be stored at -20°C and should be transported strictly under cold chain.
Histopathological	• For routine and general histopathological examination tissue pieces are to be collected in 10% formal saline (collection to be done in wide mouth bottles with10 times the volume of tissues) • A copy of detailed post-mortem report should be sent.
Toxicological (Forensic laboratory)	• For chemical analysis fresh tissues and fluids should be sent as soon as possible and on ice. Avoid addition of preservatives to the samples. Use 95% ethanol @ 1ml per gram of sample when necessary.

Preservatives used for specimen preservation

Various preservatives are used for different specimens, e.g. phosphate buffered glycerin for tissues; EDTA, sodium citrate, heparin or OCG mixture for whole blood and transport media (TPB) for swabs. The preserved specimens are most frequently transported on ice in a thermos flask or other suitable containers. The pH is adjusted to 7.2-7.4 before autoclaving. Antibiotics (Penicillin, Streptomycin, Mycostatin) are added before collection of swabs to check bacterial contamination.

3. INFORMATION TO BE SENT WITH SAMPLES

It is essential that individual samples be clearly identified using appropriate methods. Marking instruments should be able to withstand the condition of use, i.e. being wet or frozen (use indelible marking pen). Pencil has a tendency to rub off containers and labels attached to plastic will fall off when stored at -70°C. Information and case history should always accompany the samples to the laboratory, and should be placed in a plastic envelope on the outside of the shipping container. As outlined in the following section on transport of samples, this information must also be inside the shipping container. The following are suggested items that should be addressed. It would be advisable to contact the receiving laboratory to determine if it has a submission form that it would like to have submitted with the samples or if it needs other information.

i) Name and address of owner/occupier and geolocation (latitude and longitude, if available) where disease occurred, with telephone and fax numbers.

ii) Name, postal and e-mail address, telephone and fax numbers of the sender.

iii) Diseases suspected and tests requested.

iv) The species, breed, sex, age and identity of the animals sampled.

v) Date samples were collected and submitted.

vi) List of samples submitted with transport media used.

vii) A complete history would be beneficial for the laboratory and should be included if possible. Some of the components of the history are:

a) A list and description of the animals examined and the findings of the post-mortem examination.

b) The length of time sick animals have been on the farm; if they are recent arrivals, from where did they originate.

c) The date of the first cases and of subsequent cases or losses, with any appropriate previous submission reference numbers.

d) A description of the spread of infection in the herd or flock.

e) The number of animals on the farm, the number of animals dead, the number showing clinical signs, and their age, sex and breed.

f) The clinical signs and their duration including the temperature of sick animals, condition of mouth, eyes and feet, and milk or egg production data.

g) The type and standard of husbandry, including the type of feed available, possible contact with poison or poisonous plants.

h) History of foreign travel by owner or of introduction of animals from other countries or regions.

i) Any medication given to the animals, and when given.

j) Any vaccines given, and when given.

k) Other observations about the disease, husbandry practices and other disease conditions present.

4. PACKAGING AND TRANSPORT OF SAMPLES

1. Packaging

A fundamental approach is to devise a 3-layer barrier to protect the sample. The sample is placed in an appropriate **primary container** (sealed jar/bag/tube). This is then enclosed in a secondary container, which includes some adsorbent material. Note that items such as syringes, obstetrical gloves, and containers without sealable orifices are not suitable for shipment. Liquid samples should not ship in plastic

bags; a sealable jar should be used. Waterproof markers should be used when labeling specimen bags and containers: the contents and patient identification are critical information.

The **secondary container** is then placed in the shipping box (**tertiary container**), which often houses coolant packages as well as various cushioning materials (eg, polystyrene foam) to protect the sample. The coolant materials should be sealed in plastic bags to prevent condensation damage. Coolant packs should not be placed directly onto samples, such as tubes of whole blood, that could suffer adverse effects if frozen in transit. Be sure to include the suitably protected submission form. The tertiary container is ideally a sturdy polystyrene refrigerator box or a cardboard box lined with a fitted polystyrene lining. If dry ice is used, this should be noted on the cardboard box label, and the lid should not be sealed with tape. Otherwise, CO_2 released from the dry ice could increase pressure and damage the package or contents.

2. Transportation of specimens

The specimens should be forwarded to the laboratory by the fastest method available. If they can reach the laboratory within 48 hours, samples should be sent refrigerated. If dry ice is used, the additional packaging requirements must be met. Infectious substances, which can include diagnostic specimens, are not permitted to be shipped as checked luggage or as carry on luggage and must be shipped as cargo.

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